Rheedea Vol. 7(1): 15-25. 1997

Foliar epidermal features and their taxonomic significance in ten species of *Hypericum* L. (Hypericaceae)

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Abstract

The leaves in the ten species of *Hypericum* L. are simple, ovate to lanceolate. Leaf shape, size, types of stomata and their percentage, frequency fo stomata/mm^2, stomatal index have been investigated. The species *H. aegypticum, H. olympicum* and *H. montanum* are with ovate and amphistomatic leaves; *H. patulum* var. *forestii* and *H. humifusum* are with ovate and hypostomatic leaves; *H. androsaemum* and *H. patulum* are with lanceolate leaves having idioblastic sacs; *H. mysorensis* and *H. kalmanium* are with lanceolate leaves but without idioblastic sacs, and *H. empetrifolium* is with linear leaves. A key based on morphological and epidermal characters is provided for identification of the species.

INTRODUCTION

Hypericum L. (Hypericaceae) comprises 400 species distributed in the tropical and temperate regions (Willis, 1973). Various aspects like breeding systems including heterostyly and incompatibility (Noack, 1939; Salisbury, 1963; Ornduff, 1975), cytology (Robson & Adams, 1968) and palynology (Khan, 1969; Thomas, 1970; Clarke, 1975) have been investigated in species of *Hypericum*. Petal and seed morphology have been described in seven species of *Hypericum* and these were found to be of taxonomic value (Narsaiah *et al.*, 1985; Bahadur *et al.*, 1985). Although Metcalfe and Chalk (1950) described the presence of Rubiaceous type of stomata in *Hypericum*, to-date there is no further detailed study on foliar and epidermal features in any species of the genus. In the present paper foliar and epidermal features of ten speices of *Hypericum* are provided with a discussion on their utility in taxonomic considerations.

MATERIALS AND METHODS

Leaves of the 9 species of the present investigations were collected by one of us (BB) from University Botanical Gardens, Birmingham, U.K., and that of *Hypericum mysorensis* was collected (BB) from Kodaikanal, Tamil Nadu. The vouchers of slides of *Hypericum aegypticum* L., *H. androsaemum* L., *H. empetrifolium* Willd., *H. humifusum* L., *H. kalmanium* L.,

H. montanum L., *H. mysorensis* Heyne, *H. olympicum* L., *H. patulum* Thunb. *ex* Murray and *H. patulum* var. *forestii* L. are preserved in the Department of Botany, Kakatiya University. Epidermal peels from both the upper and lower surfaces were obtained by boiling the dried leaves in water. These were stained with aniline blue in lactophenol, mounted in 50% glycerine and sealed with paraffin for later observations.

Leaf shape and size; stomatal type, average stomatal size, frequency, stomatal index and shape of idoblastic sacs, sacs/mm^2, no. of sacs/leaf on both the surfaces of the leaf were investigated. Stomatal index was calculated following Cutter (1969). The measurements of different epidermal characters are given in tables 1 and 2. The terminology for the description of stomatal types is that of Metcalfe and Chalk (1950).

OBSERVATION AND DISCUSSION

LEAF MORPHOLOGY

The leaves in all the species are simple, entire, dorsiventral, epetiolate, exstipulate, glabrous on both surfaces and show opposite decussate phyllotaxy. The leaf shape is lanceolate to ovate but linear in *H. empetrifolium* with obtuse apex in *H. kalmanium* but in the rest of the species the apex is acute (Table 1).

Idioblastic sacs are present in seven of the ten species investigated with the exception of *H. empetrifolium*, *H. kalmanium* and *H. mysorensis*. Leaves are comparatively large in *H. kalmanium*, *H. patulum* var. *forestii*, *H. androsaemum*, *H. mysorensis*, *H. montanum*, *H. olympicum*, *H. humifusum*, however *H. aegypticum* and *H. empetrifolium* are with small leaves (Table 1). Thus, the leaves are morphologically distinct in the taxa studied.

EPIDERMAL FEATURES

Epidermal cell complex

The leaves in all species investigated are smooth. Epidermal cells are polygonal, 4- many sided, angular, straight walled on both the upper and lower surfaces in *H. aegypticum*, *H. androsaemum*, *H. mysorensis*, *H. olympicum*, *H. patulum* and *H. patulumforestii* (Fig. 1: A-D; Fig. 2: E-O; Fig. 3: A-F) with the exception of *H. empetrifolium* in which the lower surface is papillate (Fig. 1: E,F). The epidermal cells were found to be wavy with U to V-shaped sinuations on both the surfaces in *H. humifusum* and *H. montanum* (Fig. 1: G,H; Fig. 2: C,D) but the upper surface in *H. kalmanium* showed undulated cell walls (Fig. 2: A,B). It may be remarked that wavy nature was more pronounced on lower surface compared to the upper surface (Fig. 1: G,H; Fig. 2: A-D). Nodulated anticlinal walls were observed in all the species studied but these were absent on the lower surface of *H. empetrifolium* (Fig. 1: F). The species *H. patulum* and *H. patulum* var. *forestii* possess intercalary nodulation in their cell wall and the degree of nodulation was

Table 1. Foliar stomatal features in ten species of *Hyperium* L.

	Species	Surface	Frequency/ mm^2	Stomatal index	Stomatal size (in µm)		Stomatal type and percentage				
					Stoma	Pore	Aniso-cytic	Anomo-cytic	Para-cytic	Dia-cytic	Actino-cytic
1.	*Hypericum aegypticum* L.	US	13	21.43	23.2x19.2	14.0x4.0	+	-	-	-	-
		LS	17	27.2	22.6x20.0	14.6x4.0	+	-	-	-	-
2.	*H. androsaemum* L.	US	--	-	-	-	-	-	-	-	-
		LS	24	25.7	25.6x23.2	14.0x4.0	10	61	5	-	24
3.	*H. empetrifolium* Willd.	US	--	-	-	-	-	-	-	-	-
		LS	35	36.6	36.0x27.2	22.4x7.0	-	Sunken	Stomata	-	-
4.	*H. humifusum* L.	US	--	-	-	-	-	-	-	-	-
		LS	23	39.7	28.4x20.8	18.2x4.0	45	32	23	-	-
5.	*H. kalmanium* L.	US	--	-	-	-	-	-	-	-	-
		LS	11	28.6	26.8x19.2	15.6x4.4	23	21	56	-	-
6.	*H. montanum* L.	US	3	4.5	29.2x26.6	19.3x4.6	64	36	-	-	-
		LS	18	36.84	26.0x20.4	14.4x4.4	81	19	-	-	-
7.	*H. mysorensis* Heyne	US	38	32.6	18.0x19.2	12.0x5.0	+	-	-	-	-
		LS	23	19.0	18.8x18.8	12.4x5.0	+	-	-	-	-
8.	*H. olympicum* L.	US	4	10.4	26.0x25.2	16.4x4.4	8	6	86	-	-
		LS	12	31.1	23.2x23.6	13.6x4.0	25	12	63	-	-
9.	*H. patulum* Thunb. *ex* Murray	US	--	-	-	-	-	-	-	-	-
		LS	16	25.7	28.4x24.0	15.6x4.0	50	41	9	-	-
10.	*H. patulum* var. *forestii* Chittenden	US	--	-	-	-	-	-	-	-	-
		LS	23	23.5	24.4x20.8	14.0x4.4	6	76	2	-	16

US = Upper surface LS = Lower surface

comparatively less on the upper surface in *H. patulum* compared to all other species studied. It is interesting to point out that the nodulation in the specieswith wavy walls was found to be pronounced at either ends of U or V-sinuation (Fig. 1: G-H; Fig. 2: A-D). In general, the epidermal cells are smaller in size and more in number on the lower surface of the leaf than on the upper surface in most of the species except in *H. mysorensis*.

Solitary and prismatic calcium oxalate crystals were recorded on both the surfaces in all species investigated except in *H. empetrifolium* (Fig. 1: E,F). Although the crystals are solitary, four types of crystals were observed depending on their shape and structure. They are: round, ovoid, spindle-shaped, and round but prismatic type. The crystals were found to be of the same type on both the surfaces while in *H. mysorensis* rounded-prismatic crystals were recorded on the upper surface, whereas rounded to ovoid and prismatic crystals occur on lower surface of the leaf (Fig. 2: E,F). Spindle-shaped crystals were observed on both the surfaces in *H. kalmanium* (Fig. 2: A,B) and all the four crystal types found were recorded in *H. montanum* (Fig. 2: C,D). Distribution of crystals in the remaining species was noted on both the leaf surfaces in *H. aegypticum, H. androsaemum, H. olympicum* (round and ovoid, Fig. 1: A-D; Fig. 3: A,B), *H. humifusum* (round, ovoid and spindle-shaped), and *H. patulum* var. *forestii* (round, ovoid and prismatic, Fig. 3: C-F).

Stomatal Complex

The data on stomatal features in species of *Hypericum* studied are summarised in Table 1. The species *H. aegypticum, H. montanum, H. mysorensis* and *H. olympicum* are amphistomatic while rest of the species studied are hypostomatic. Anisocytic stomata (100%) have been observed in *H. aegypticum* and *H. mysorensis* but in the remaining species the following predominant stomatal types on both the surfaces were observed: anisocytic (*H. humifusum, H. montanum, H. patulum*), anomocytic (*H. androsaemum, H. empetrifolium, H. patulum* var. *forestii*), and paracytic (*H. kalmanium, H. olympicum*; see Table 2). Actinocytic giant stomata with 6-12 subsidiaries has been found in *H. androsaenum* (Fig. 2: O) and *H. patulum* var. *forestii*. Actinocytic stomata are larger (29.0 x 20.0 μm) compared to the normal stomata.

High stomatal frequency/mm^2 and stomatal indices have been observed on lower surface than on the upper surface in all amphistomatic species except *H. mysorensis* where it was reverse. Higher stomatal frequency among the hypostomatic species was recorded in *H. empetrifolium* followed by *H. humifusum, H. patulum* var. *forestii, H. patulum* and *H. kalmanium* (Table 2). On the whole it was noted that the highest frequency on the upper surface is in *H. mysorensis* and lowest on the upper surface is in *H. montanum*. Stomata occur rarely on either side of the midvein in the latter species.

The stomatal index among the hypostomatic as well as amphistomatic species is highest in *H. humifusum*. Larger stomata were observed on the upper surface in all amphistomatic species except in *H. mysorensis* in which the stomata on both the surfaces was almost equal and the largest stomata have been found in *H. empetrifolium*.

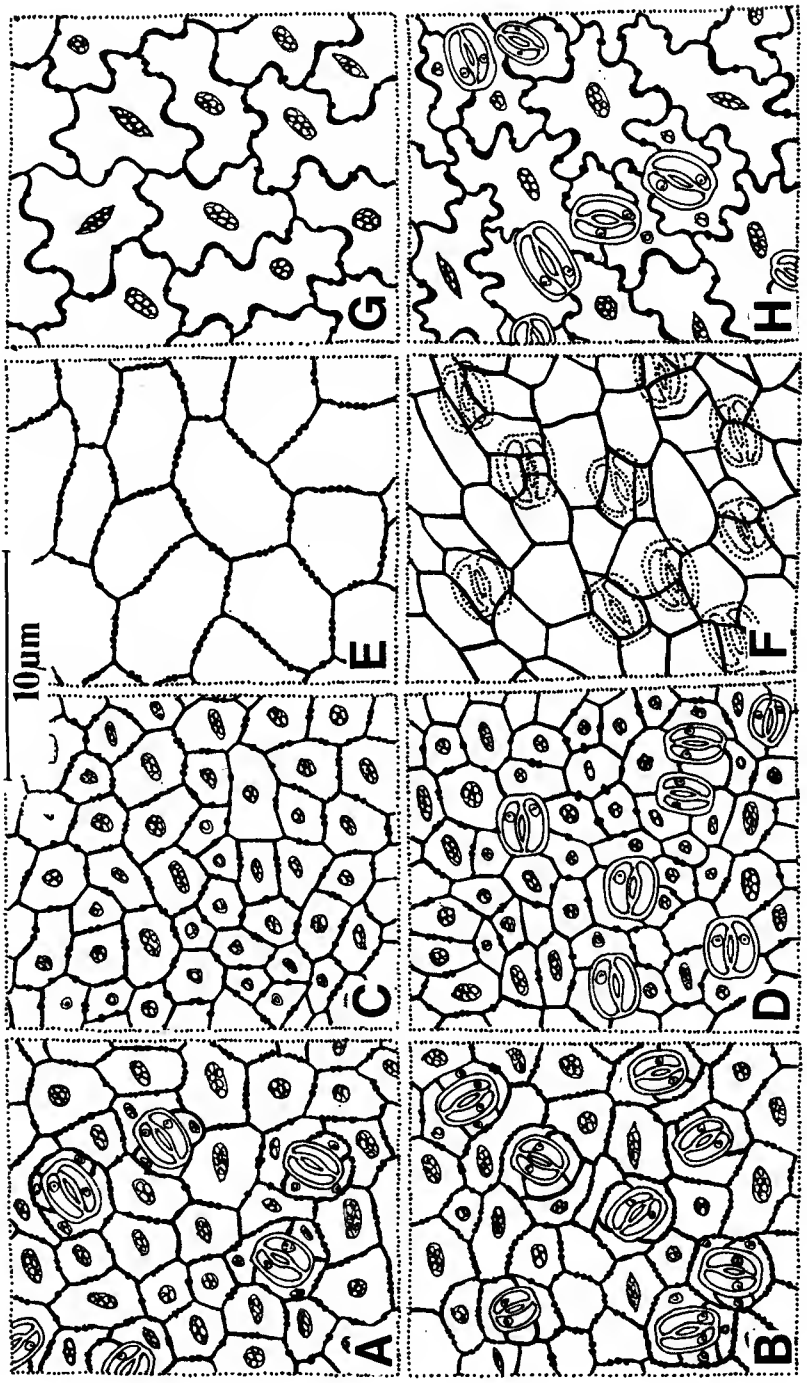

Fig. 1: A-H. Upper and lower leaf surfaces of *Hypericum* species. A,B. *H. aegypticum*; C,D. *H. androsaemum*; E,F. *H. empetrifolium* (note the sunken stomata on lower surface); G,H. *H. humifusum*.

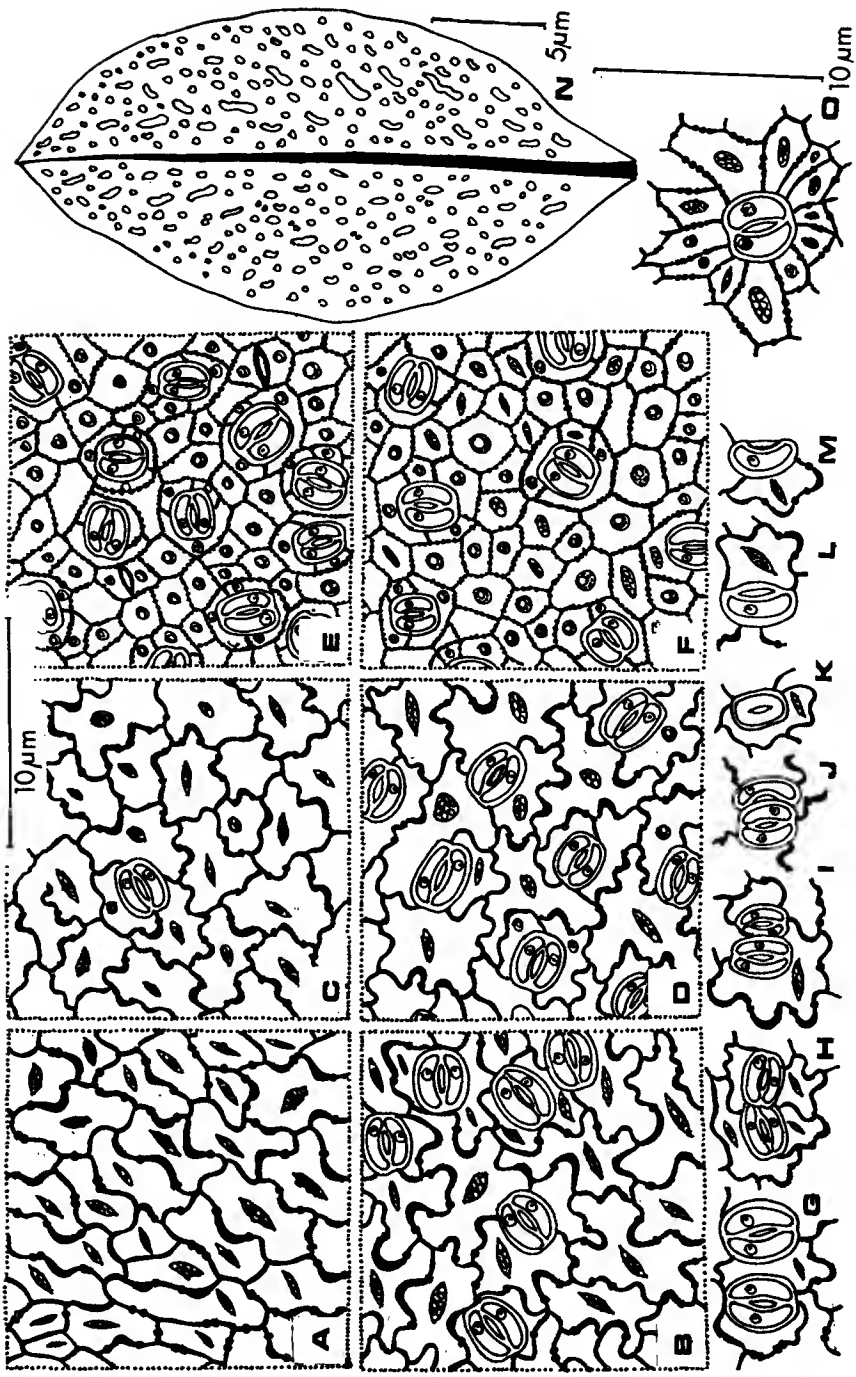

Fig. 2: A-F: Upper and lower leaf surfaces of *Hypericum* species, A,B. *H. kalmianum*; C,D. *H. montanum*; E,F. *H. myrsinites*; G-M. Abnormal stomata in *H. kalmianum*; G. Common subsidiary cell; H. Pole to pole contiguous stomata; I. Side to side contiguous stomata; J. Contiguous stomata with single guard cell; K. Without demarkation of guard cells; L. Pore with single guard cell; M. Hall stoma; N,O. Leaf of *H. androsaemum*; N. Various shapes of idioblastic sacs; O. Actinocytic stoma.

It is interesting to point out that *H. olympicum* is characterised by stomatal ledges while sunken stomata occur in *H. empetripolium*, and some of the anomocytic stomata in *H. montanum* and *H. olympicum* tend to be anisocytic.

Abnormal Stomata

Most of the species studied showed various types of stomatal arrangement. Pole to pole arrangement occur in *H. androsaemum* (3%), *H. humifusum* (3%), *H. patulum* (1.5%, Fig. 3.1), *H. patulum* var. *forestii* (1%) and *H. kalmanium* (2.5%, Fig. 2: H); side to side stomata occur in *H. humifusum* (3.5%) and *H. kalmanium* (Fig. 2: I). Stomata with common subsidiary cell, contiguous stomata with single guard cell, stomata without demarcation of guard cells, without pore and single guard cell have been observed in *H. kalmanium* (Fig. 2: G,J-M). Stomata with transverse cytoplasmic connections and single guard cell with simple connection have been found in *H. olympicum* (Fig. 3: G,H); common subsidiary cell with lateral cytoplasmic connections and without stoma but with subsidiaries were observed in *H. patulum* (Fig. 3: J,K); and in *H. mysorensis* degenerating stomata were also observed (Fig. 2: E,F).

Idioblastic Sacs

Light yellow and translucent dots, "the idioblastic sacs" were observed in the leaves of seven species of *Hypericum*. These can be easily seen by holding the leaves against light, but with a pocket lens these are more clearly seen. The idioblastic sacs are round, ovoid to oblong and linear in outline, and appear as large cavities and show granular contents (Fig. 2: N; Fig. 3: L,M). As shown in Fig. 3: M, the idioblastic sac has an inner ring of eight narrow rectangular cells and an outer ring of 9-10 larger cells which are externally connected by 12-14 epidermal cells. These are absent in *H. empetrifolium*, *H. kalmanium* and *H. mysorensis*. Number of idioblastic sacs/leaf and per unit area (mm^2) were also observed. *H. patulum* showed highest number of 839/mm^2 and *H. humifusum* with 258/mm^2; whereas in *H. montanum* these are fewer. The idioblastic sacs are dimorphic being round and linear in *H. androsaemum*, *H. patulum* and *H. patulum* var. *forestii*, whereas monomorphic sac - either round to spheroidal - occur in four of the ten species investigated. Round idioblastic sacs were larger in *H. olympicum* followed by *H. humifusum*, *H. montanum* and *H. aegypticum* (Table 1). The idioblastic sacs which are longer than their breadth with their tips swollen were observed in *H. patulum*, *H. patulum* var. *forestii* and *H. androsaemum* (Fig. 2: N; Table 1). Similar observations were made in *Cleome aspera* by Rajagopal and Ramayya (1968) and in 66 species of Malvales by Shanmukha Rao and Ramayya (1984).

From the foregoing, it is obvious that both the morphological and epidermal characters of the leaves in ten species of *Hypericum* studied are taxonomically valuable and accordingly a key is proposed as follows for the identification of the species.

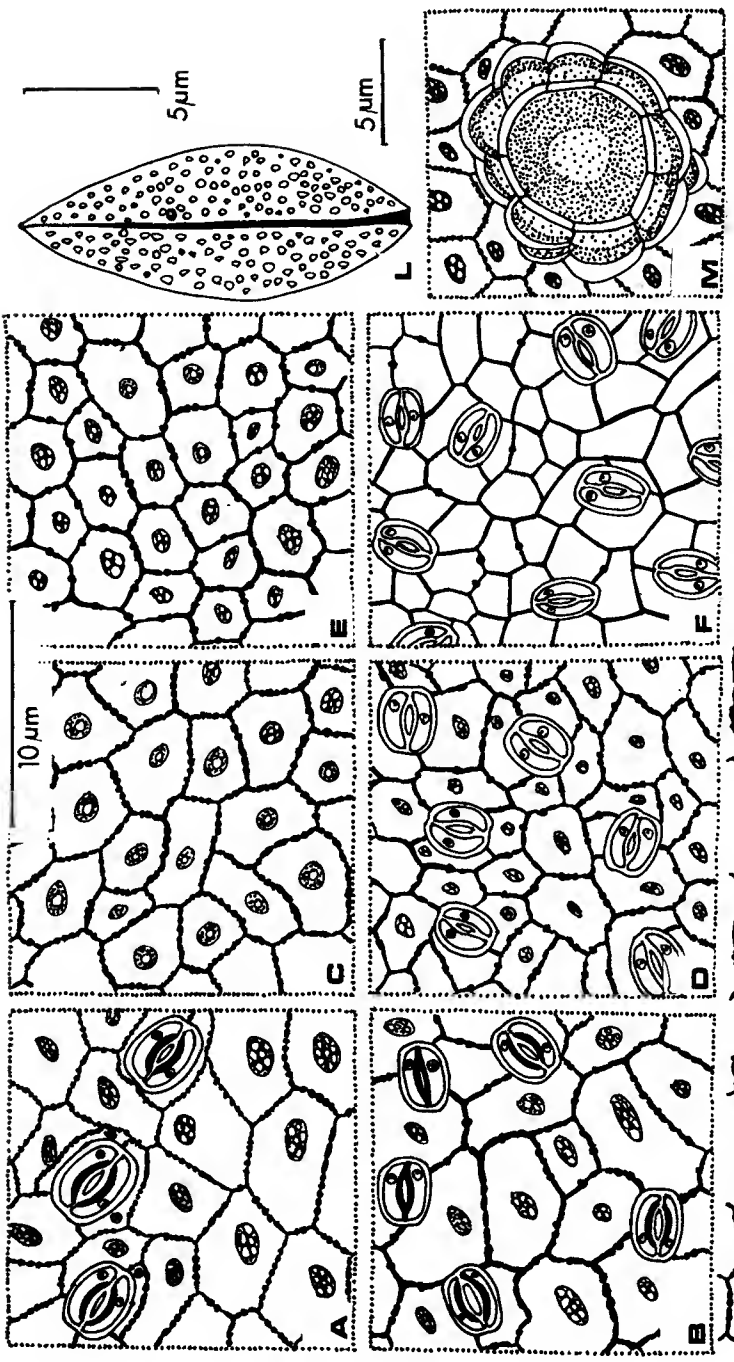

Fig. 3: A-F. Upper and lower leaf surfaces of *Hypericum* species. A,B. *H. olympicum*; C,D. *H. patulum*; E,F. *H. patulum* var. *forestii*; G-K. Abnormal stomata in *H. olympicum* (G-H) and *H. patulum* (I-K); L-M. Leaf showing idioblastic sacs in *H. olympicum* (L) and one idioblastic sac enlarged (M).

Foliar epidermal features in *Hypericum* L.

Key to the species based on foliar epidermal features

1. Leaves with idioblastic sacs.
   2. Leaf lamina ovate; amphistomatic or hypostomatic; epidermal cells angular or wavy.
      3. Leaves amphistomatic.
         4. Epidermal cells angular; walls straight, 4 to many sided, polygonal; stomata anisocytic or paracytic.
            5. Stomata anisocytic ..... *H. aegypticum*
            5. Stomata predominantly paracytic ..... *H. olympicum*
         4. Epidermal cells wavy with U-shaped sinuations; stomata predominantly anisocytic ..... *H. montanum*
      3. Leaves hypostomatic.
         6. Epidermal cells angular, walls straight, 4 to many sided, polygonal; stomata predominantly anomocytic ..... *H. patulum* var. *forestii*
         6. Epidermal cells wavy with U-shaped sinuations; stomata anisocytic ... *H. humifusum*
   2. Leaf lamina lanceolate; hypostomatic; epidermal cells angular, walls straight, 4 to many sided, polygonal.
      7. Stomata predominantly anomocytic ..... *H. androsaemum*
      7. Stomata both anomocytic and anisocytic ..... *H. patulum*
1. Leaves without idioblastic sacs.
   8. Leaf lamina lanceolate, amphistomatic or hypostomatic.
      9. Leaves amphistomatic; epidermal cells angular, walls straight, 4 to many sided, polygonal; stomata anisocytic..... *H. mysorensis*
      9. Leaves hypostomatic; epidermal cells wavy with U-shaped undulations; stomata predominantly paracytic ..... *H. kalmanium*
   8. Leaf lamina linear; leaves hypostomatic; epidermal cells angular, walls straight, polygonal on upper surface, papillate on lower surface with sunken stomata ..... *H. empetrifolium*

Table 2. Foliar and idioblastic sac character in ten species of *Hypericum* L.

Sl. No.	Species	Lamina size (in cms) L x W	No. of Idioblastic sacs/Leaf		Idioblastic sacs mm^2		Shape and size of Idioblastic sacs (in µm)	
			Round	Linear	Round	Linear	Round	Linear
1.	*H. aegypticum* L.	1.2x0.41 (1.1-1.3x0.3-0.5)	571 (541-593)	--	25 (21-29)	--	35.82x32.63 (23.88-47.76 x 19.90-47.76)	
2.	*H. androsaemum* L.	2.5x1.22 (2.4-2.6x1.1-1.3)	118 (112-125)	395 (352-446)	6 (3-10)	5 (2-7)	116.4x108.6 (54.3-181x 54.3x181)	316.75x95.93 162.9-814.5x 72.4-126.7)
3.	*H. empetrifolium* Willd.	0.44x0.18 (0.4-0.5x0.15-0.20)	---	---	---	---	--	--
4.	*H. humifusum* L.	1.3x0.6 (1.1-1.5x0.5-0.7)	258 (237-272)	--	11 (8-14)	--	75.62x71.64 (19.90-127.36x19.90-07.46)	
5.	*H. kalmanium* L.	7.3x4.2 (7.0-7.5x4.0-4.4)	--	--	--		--	--
6.	*H. montanum* L.	1.9x0.86 (1.6-2.3x0.70-0.91)	265	--	7	--	75.62x69.65 (39.8-107.46x39.8-107.46)	
7.	*H. mysorensis* Heyne	2.5x0.62 (2.5-2.6x0.5-0.62	--	--	--	--	--	--
8.	*H. olympicum* L.	1.44x0.54 (1.4-1.5x0.5-0.6)	418 (375-454)	--	13 (11-20)	--	101.2x98.0 (60-168x60-168)	
9.	*H. patulum* Thunb. *ex* Murray	3.84x1.26 (3.7-4.0x1.2-1.3)	546 (509-575)	293 (268-318)	16 (12-24)	3 (2-6)	32x29.2 (2.4-40x24-(144.8-1176.5x40)	522.9x64.4 54.30-90.5)
10.	*H. patulum* var. *forestii* Chittenden	3.48x1.72 (3.4-3.6x1.7-1.8)	430 (402-455)	386 (376-395)	39 (28-52)	7 (5-11)	29.2x28.8 (12-40x 12-40)	479.3x92.2 (162.9-1176.5x 54.3-126.7)

(L= Length; W = Width; Idiobl. = Idioblastic sacs. Figures in Parentheses represent range of variation)

Acknowledgement

We thank the Curator, University Botanical Garden, Birmingham University, U.K., for providing the leaf materials.

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